THE DEMONSTRATION OF TWO CHROMATOPHOROTROPICALLY ACTIVE SUBSTANCES IN THE LAND ISOPOD, TRACHELIPUS RATHKEI

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The remarkable ability of decapod Crustacea to undergo adaptive and rhythmic color changes has been under critical study for many years. Early investigators were concerned primarily with the structure of the chromatophores and with the possibility of neural control of pigment migration. Koller (1925), working with *Crago*, gave the first clear cut evidence for humoral control of the chromatophores. Perkins (1928) with *Palaemonetes* and Koller (1928) with *Crago* determined that the substance responsible for these effects was elaborated by some tissue of the eyestalk. Hanstrom (1937) successfully demonstrated that the observed chromatophorotropic activity of the eyestalk was localized in the region of the sinus gland. The extirpation experiments of Brown and Cunningham (1939) and Brown, Ederstrom and Scudamore (1939) presented conclusive evidence of the role of the sinus gland as a source of chromatophorotropins. With the exception of *Crago*, the decapods studied would seem to fall into one of two main categories: (1) those resembling *Palaemonetes* in that sinus gland extracts exert a concentrating influence upon the predominant pigment type and (2) those resembling *Uca* in that extracts of the sinus gland produce a dispersion of the dark pigment.

The presence of chromatophorotropins within the central nervous system was first shown by Brown (1933) for *Palaemonetes*. Subsequent work has established the presence of chromatophore activators within the central nervous system of most decapods. These factors have been found in some cases to have the same action as the hormones of the sinus gland, and in other instances they act antagonistically to them (Knowles, 1939; Brown and Ederstrom, 1940; Brown and Wulff, 1941; Brown, 1950; Sandeen, 1950; Enami, 1951; Brown, Webb and Sandeen, 1952).

The ability of isopod Crustacea to exhibit color responses has long been established and yet the exact nature of the mechanisms controlling these responses has not been clearly demonstrated. The presence of two hormones, one producing a dispersion of the melanin and the other a concentration of the melanin, was indicated in *Ligia oceanica* by the unique experiments of Smith (1938). However, Okay (1945) working with *Idothea baltica* and *Sphaeroma serratum* reported that the responses observed were dependent upon the presence of a single hormone.

Studies on the effects of extracts of isopod heads have yielded apparently conflicting results. In general, extracts prepared from isopod heads when injected into isopods of the same or related species have produced a concentration of the melanin (Kleinholz, 1937; Okay, 1945; Suneson, 1947; Carstam and Suneson, 1949).

Stahl (1938) has shown a dispersion of the red pigment of the decapod

Leander adspersus following the injection of extracts of the entire heads of the isopods *Mesidothea, Porcellio* and *Oniscus* and a concentration of the same pigment following the injection of extracts of the heads of *Idothea baltica.* Carstam and Suneson (1949) observed both a dispersing and a concentrating effect upon the red pigment of *Leander* following injection with extracts of the heads of *Idothea neglecta.*

Evidence indicating the presence of structures in *Oniscus asellus* which are apparently homologous to the sinus glands of other Crustacea (Walker, 1935; Stahl, 1938) has suggested the advisability of further investigations of the chromatophorotropins of isopod Crustacea. In addition, Gabe has shown in *Oniscus asellus* (1952a) and in *Sphaeroma serratum* (1952b) histological variations in the sinus glands during the intermolt cycle suggesting the physiological intervention of the gland in this cycle. However, its role in color change was not considered.

The wide variations in color observed in certain species of terrestrial isopods seemed to indicate the possibility that the progressive fixation of pigment, occurring as an adaptation to the terrestrial environment, may not be complete in these forms.

The purpose of this investigation was to determine the degree of chromatic activity occurring within these isopods and, if possible, to cast some light on the nature and distribution of the chromatophorotropins involved in these responses.

The authors would like to acknowledge their gratitude to John R. Cortelyou for his suggestion of the problem and his keen interest and aid throughout the course of this work.

Materials and Methods

Specimens of *Trachelipus rathkei,* a terrestrial isopod, were collected in large numbers from wooded areas in the vicinity of Chicago, Illinois. The animals when collected exhibited wide variations in color ranging from a mottled yellow-brown to an intense black. Males were normally much darker than females. Stock supplies of animals were maintained in covered battery jars containing moistened pieces of decaying wood. These conditions allowed for the successful maintenance of the stock in the laboratory for two to three months. Animals to be light-adapted were placed into clear Petri dishes lined with moistened filter paper. The dishes were then kept on a white background under constant overhead illumination. Animals to be dark-adapted were maintained in a similar manner in Petri dishes painted with black enamel. Attempts were made to establish a relatively constant humidity within the dishes, through the addition of water to the filter paper.

1. Animals

Microscopic examination of the pigmentary system of adult *Trachelipus* reveals that the coloration of the animal is dependent upon the relative distribution of two types of pigment within an apparently syncytial network of chromatophore processes. Although, in many instances, each pigment would appear to be contained within a distinct chromatophore network, the yellow usually lying beneath the black, the presence of extensive dichromatic areas indicates that the syncytium

is complete. Observations of the external surface of the intact dorsal segments showed a cuticle pattern which did not permit clear identification of the chromatophore structure. On the other hand, the internal surface is obscured by a considerable amount of adherent muscle. Histological preparations shed little light on this problem, since the yellow pigment is soluble in acid fixatives and the decalcifying agents used for these studies. At this time, no definite conclusions can be drawn as to the exact structural nature of the pigmentary system of the adult *Trachelipus*.

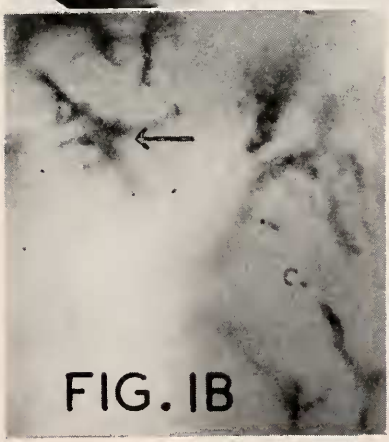
However, observations of larval *Trachelipus* prior to and immediately following their release from the brood pouch of the female show that in this species there are initially discrete chromatophores (Fig. 1) which rapidly develop into syncytial networks. These appear to be dichromatic and retain this condition during the early stages of fusion. Unfortunately, culture methods were not adequate to maintain the young animals long enough to observe the subsequent development of the chromatophore network.

The nervous system of *Trachelipus* is typically crustacean in nature and appears to be identical to that described for *Oniscus asellus* Linneas (Walker, 1935). The system is relatively unspecialized. The division of the crustacean brain into protocerebrum, deuterocerebrum and tritocerebrum is only weakly apparent in these forms. The protocerebral ganglia are clearly defined and are continuous with the well developed optic tracts. At the distal end of each optic tract in the region of the lamina ganglionaris, there is attached in a slightly ventrolateral position, a small oval structure which is bluish-white when viewed with reflected light. Upon injury this structure is seen to release a bluish viscous fluid, which diffuses through the saline. Both from its morphological appearance and its topographical relationship it resembles the sinus glands of other Crustacea (Fig. 2) and is referred to as such in the subsequent discussion. The deuterocerebrum is greatly reduced and appears as a narrow strip lying behind the protocerebral ganglia. The tritocerebrum, which bears the nerves leading to the antennae, is continuous with the short circumesophageal connective on each side. No tritocerebral commissure was observed although it may have been lost during the dissection. The subesophageal ganglion is not clearly defined and includes the first and second maxillary ganglia and the maxipedal ganglion. The nerve cord is characterized by a uniformity of segmentation of the thoracic ganglia and an anterior fusion of abdominal ganglia. The abdominal nerve cord thus consists merely of the posteriorly directed nerves arising from the fused ganglia.

2. Preparation of extracts and method of assay

For the preparation of extracts, the entire nervous system was removed from the animal with the aid of a dissecting microscope ($\times$ 45), and transferred to a syracuse watch glass containing Van Harreveld's solution. Animals from which the nervous systems were taken ranged in length from 9 to 11 mm. Care was taken to remove the entire system in one piece, such that the sinus glands were removed from the animal while retaining their connection to the optic tracts. The surrounding tissue was dissected away in order that extracts would include only the tissue of the sinus glands or of the neural elements.

To determine the relative distribution of the chromatophorotropically active



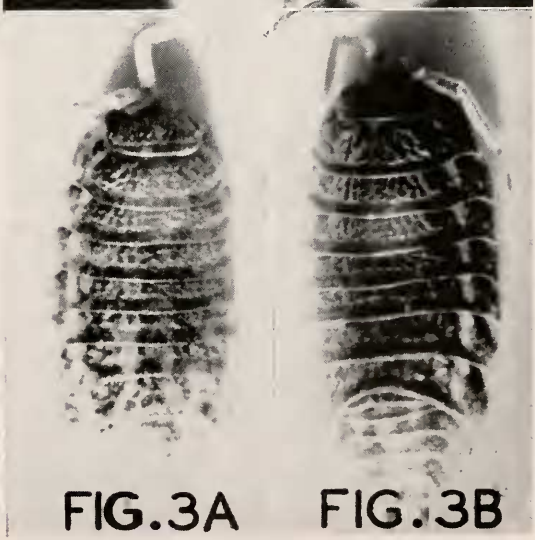


FIGURE 1. Discrete chromatophores of larval *Trachelipus*. (A) × 150; (B) × 440, illustrating early stages of concentration in response to light stimulus.

FIGURE 2. Dissected head of *Trachelipus* demonstrating sinus glands and cerebral region. (× 45)

FIGURE 3. Responses of *Trachelipus* to light. Adult female *Trachelipus* after being maintained in constant (diffuse overhead) illumination while on a light background (A), and after being maintained in total dark (B). (× 13.5)

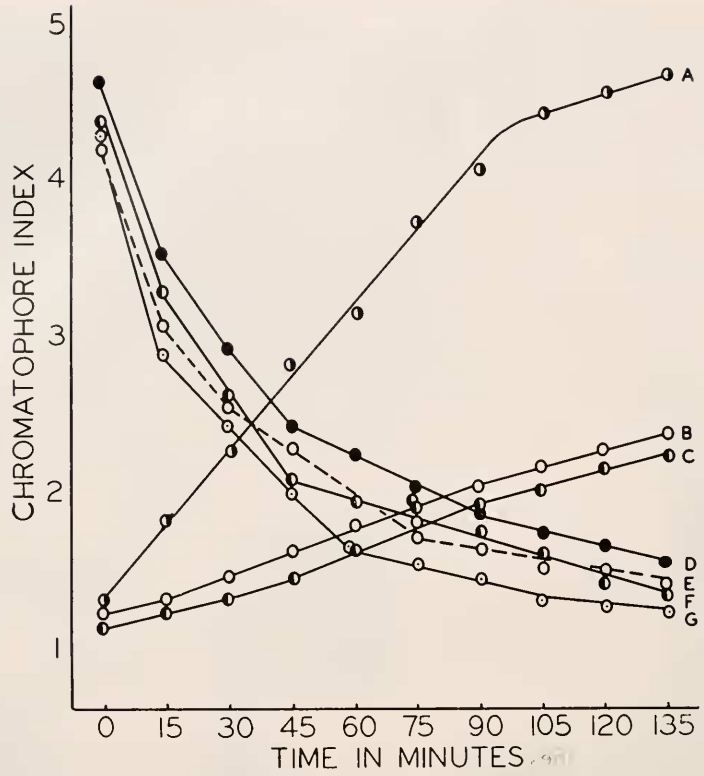


FIGURE 4. Comparative activities of extracts prepared the sinus glands and neural elements of *Trachelipus* when assayed upon the red chromatophores of *Cambarus* (concentration 5). Dispersing activity: sinus glands (A); optic tracts (B); cerebral ganglia (C). Concentrating activity: subesophageal ganglia (D); anterior half of thoracic cord (E); connectives (F); posterior half of thoracic cord (G).

substances, extracts were prepared from nervous systems which had been divided into the following regions: (1) sinus glands; (2) optic tracts; (3) cerebral ganglia (including deuterocerebrum and protocerebrum); (4) circumesophageal connectives (including the tritocerebrum); (5) subesophageal ganglia (including first and second maxillary and the maxipedal ganglia); (6) anterior half of thoracic cord; (7) posterior half of thoracic cord (including the abdominal ganglion). Due to the extreme fragility of the abdominal nerves, no determinations were made of the activity of these elements. The individual fragments were placed into separate mortars. Because the chromatophorotropins undergo a slow inactivation at room temperature, the mortars were placed on ice during the preparation of the extracts. When 10 intact nervous systems had been dissected and divided into the appropriate regions, the fragments were triturated and mixed with one cc. of saline. The extracts were then maintained in small capped vials and refrigerated until further use. Extracts could be kept in this manner for several days without undergoing a loss of activity.

For use in experiments designed to determine the relative effects of concentration, the extracts were prepared in the same manner, from nervous systems divided into two portions, the first of which included the sinus glands, optic tracts and cerebral ganglia; the second included the connectives, subesophageal ganglion and entire thoracic cord. The number of nervous systems used in the preparation of these extracts varied according to the experiment and will be discussed with the appropriate results. In specified instances, extracts were prepared from entire nervous systems, including the sinus glands.

Since boiled extracts showed no significantly marked change in activity, the extracts in most cases were not boiled.

To alleviate the technical difficulties involved in observations of the pigmentary system of *Trachelipus*, assays were made upon isolated portions of the carapace of *Cambarus* sp. The carapace was removed from the animal and divided into pieces approximately 5 × 5 mm. in size. Since the effect upon the chromatophores seemed to proceed from the periphery to the center of the piece, more uniform results were insured by using pieces of approximately the same size. These were then placed into hanging drop slides containing a known concentration of extract. Into each depression were placed pieces of the carapace removed from: (1) a light-adapted animal; (2) an animal whose eyestalks had been removed at least 12 hours before the experiment was begun. In this way, the effectiveness of each extract was determined upon chromatophores which were initially fully dispersed as well as upon those which were completely concentrated. Controls consisting of carapace fragments maintained in Van Harreveld's solution were used in each experiment. The condition of the red chromatophores was then determined at 15-minute intervals using a standard chromatophore index (Slome and Hogben, 1928) where 1 signifies maximum concentration of pigment and 5 maximum dispersion with 2, 3, and 4 indicating the intermediate conditions. Since a difference was noted in the reaction of the chromatophores, average values were determined. Because this difference was more pronounced in older animals, small crayfish having a carapace length of 1.5 to 2.5 cm. were used. Each individual experiment was repeated four to five times.

Extracts of *Cambarus* sinus glands and central nervous systems were used to indicate that the chromatophores of isolated pieces of carapace retain their activity for several hours.

In another series of experiments extracts were injected into young intact *Cambarus*. One type of extract included sinus glands, optic tracts, and cerebral ganglia, while a second consisted of connectives, subesophageal ganglia and thoracic cords. The individuals receiving these extracts were (1) light-adapted for 24 hours and (2) eyestalkless, prepared 24 hours before the time of injection.

Experiments and Results

1. Responses to light

Adult *Trachelipus* exhibit slow and weak chromatic responses to light. Animals maintained upon a light background under the stimulus of diffuse light become slightly but perceptibly lighter than animals maintained in the dark (Fig. 3). More intense illumination, however, results in a darkening of the animals, even

upon a white background. In these cases the additional factor of an increase in temperature was also involved.

The discrete chromatophores of larval *Trachelipus* exhibit rapid and strong responses to light. Under the stimulus of light, the pigment of these cells rapidly concentrates. Although in many instances this results in the typically punctate condition of the chromatophores, in several cases the pigment apparently concentrated within the chromatophore processes, thus presenting a beaded condition. This reaction to light declines rapidly with age, in correlation with the progressive fusion of the chromatophore network.

2. Presence and distribution of chromatophorotropins

To determine the presence and relative distribution of the chromatophorotropic factors, extracts were prepared of sinus glands alone, as well as of isolated pieces of the central nervous system, as described in the preceding section. Since 0.5 cc.

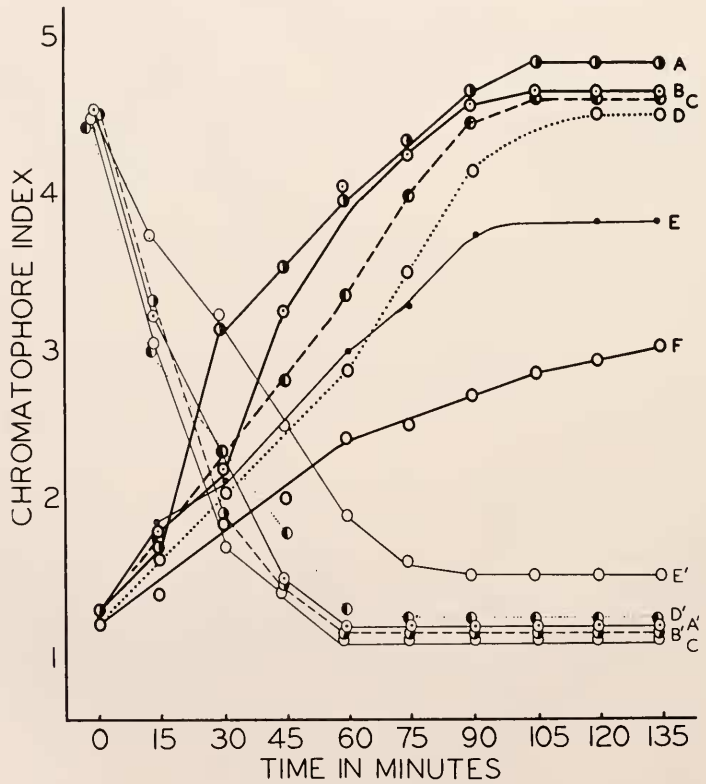


FIGURE 5. Effect of changes in concentration on the rate and magnitude of responses of the red chromatophores of *Cambarus* induced by extracts prepared from the sinus glands, optic tracts and cerebral ganglia of *Trachelipus*. (A—conc. 15; B—conc. 10; C—conc. 5; D—conc. 2.5; E—conc. 0.5; F—conc. 0.25.) Similar effects induced by extracts prepared from the connectives and thoracic cords of *Trachelipus*. (A'—conc. 15; B'—conc. 10; C'—conc. 5; D'—conc. 2.5; E'—conc. 0.5.)

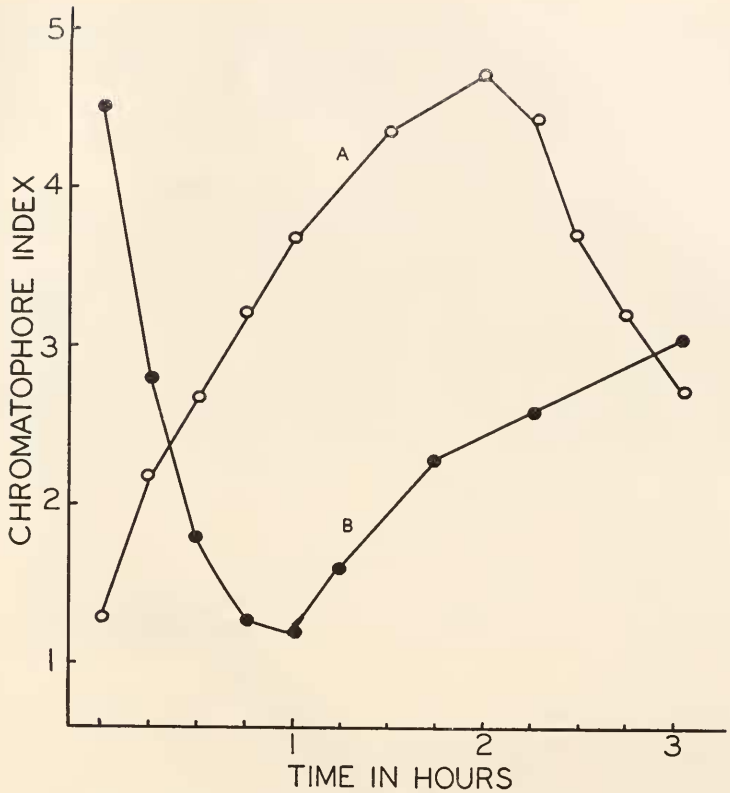


FIGURE 6. Average response of eight young *Cambarus* to injected extracts of (A) sinus glands, optic tracts, and cerebral ganglia in a conc. of 2.5; (B) connectives and thoracic cord in a conc. of 2.5.

of each extract was assayed upon isolated pieces of the carapace of *Cambarus,* the effective concentration included the specified structures from five animals.

An average of the results obtained from six identical experiments are presented in Figure 4. It can be seen that the sinus glands contain a substance which induces a dispersion of the red pigment of *Cambarus.* Whether the weak dispersing action of optic tracts and cerebral ganglia is due to the active production of this factor or merely to a diffusion through the tissue could not be determined by the method employed.

A similar number of experiments using extracts of the connectives or any segment of the thoracic cord demonstrated a concentration of the red pigment (Fig. 4). Since the individual fragments elicted approximately the same responses the substance controlling the concentrating action would seem to be relatively uniform in its distribution throughout the various regions of the cord.

3. Effect of concentration

In order to determine the relative effectiveness of the two chromatophorotropic factors, assays were made of various concentrations of the organs demonstrated

to contain each factor. Since 0.5 cc. was used in each assay, an extract prepared from the structures of a single animal will be designated as having a concentration of 1. Extracts were prepared of the sinus glands, optic tracts and cerebral ganglia in effective concentrations of 15, 10, 5, 2.5, 0.5, and 0.25. Another series of extracts was prepared and included connectives and thoracic cords in effective concentrations of 15, 10, 5, 2.5, and 0.5.

The results obtained from the two series of experiments are shown in Figure 5. It can be seen that above a concentration of 2.5 there is very little increase in the activity of either the dispersing or the concentrating factor. Increasing the concentration above this point results in a slightly increased rate of activity while the magnitude of the response is not appreciably altered in either case. Decreasing the concentration below this point, however, results in a decrease in both the rate and magnitude of response. Although variations in the potency of extracts at low concentrations cannot be completely explained at this time, it is possible that extracts prepared from light-adapted animals contained slightly more of the factor inducing dispersion than did extracts prepared from dark-adapted animals.

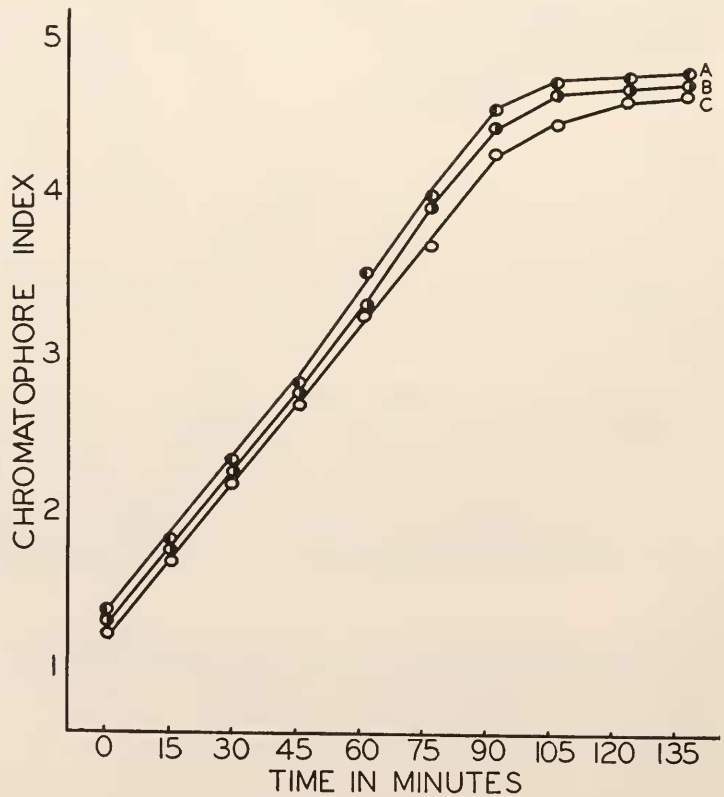


FIGURE 7. Comparative dispersing activity of extracts prepared from: A—sinus glands and entire nervous systems; B—sinus glands, optic tracts and cerebral ganglia; C—sinus glands alone. All extracts were prepared in a concentration of 5.

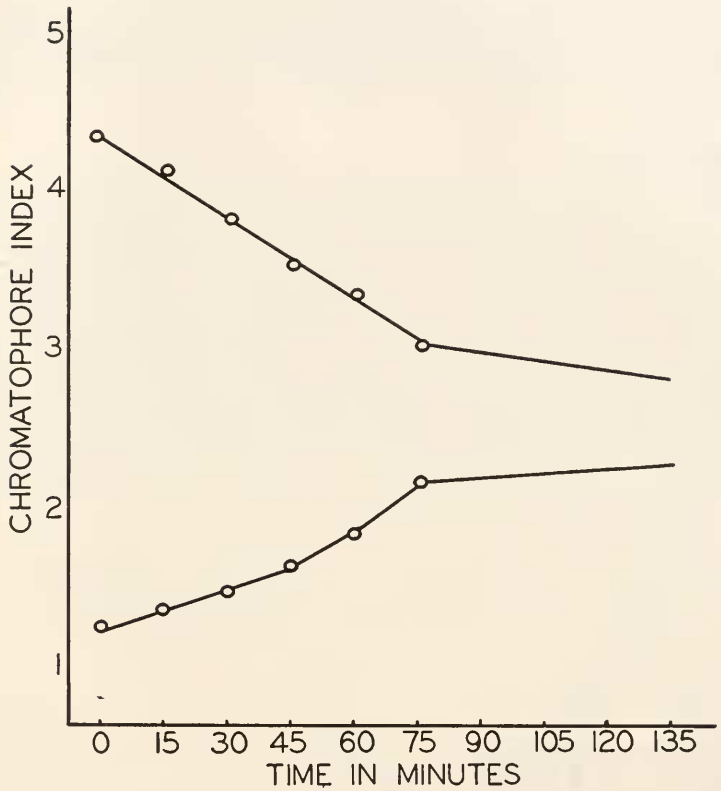


FIGURE 8. Dual response of the red chromatophores of *Cambarus* following perfusion with dilute (conc. 0.25) extracts of sinus glands plus the entire nervous system of *Trachelipus*.

A comparison of the two families of curves obtained (Fig. 5) indicates that the potency of the dispersing factor decreases more rapidly with decreasing concentrations that does that of the concentrating factor. A difference in the rate of activity of the two factors was also noted, since, within the range of concentrations studied, maximum dispersion was never attained in less than 90 minutes while complete concentration could be achieved in less than one hour. These two observations suggest that the factor eliciting concentration is stronger than that inducing dispersion. However, when extracts prepared from the entire nervous system including the sinus glands were assayed, only a dispersion of the red pigment was achieved. These conflicting observations suggest the existence of an antagonistic action between the two factors.

Since these studies, made upon isolated pieces of the branchiostegite, gave no indication of the duration of the responses obtained, eight young intact *Cambarus* were injected. Light-adapted *Cambarus* were injected with an extract of sinus glands, optic tracts and cerebral ganglia, while eyestalkless *Cambarus* were injected with extracts of connectives and thoracic cords. Both extracts were prepared in a concentration of 2.5. The responses obtained (Fig. 6) are of es-

sentially the same rate and magnitude as had previously been attained by the chromatophores of isolated pieces of the carapace. With respect to duration, the concentrating factor appeared to have a slightly more lasting effect than did the dispersing factor.

4. Antagonistic action

In sections 2 and 3 it was demonstrated that extracts of the sinus glands alone, or of the sinus glands, optic tracts and cerebral ganglia, induced only a dispersion of the red chromatophores of *Cambarus* while extracts of the connectives and thoracic cord induced a concentration of the same pigment. It was also shown that extracts of the entire nervous system (including the sinus glands) produced only a dispersing effect. The comparative dispersing action of the three types of extracts containing sinus glands, in a concentration of 5, is shown in Figure 7. These responses indicate that the concentrating factor in the nerve cord is prevented from eliciting a response when in the presence of high titers of the dispersing factor of the sinus gland. It is also apparent that the nervous

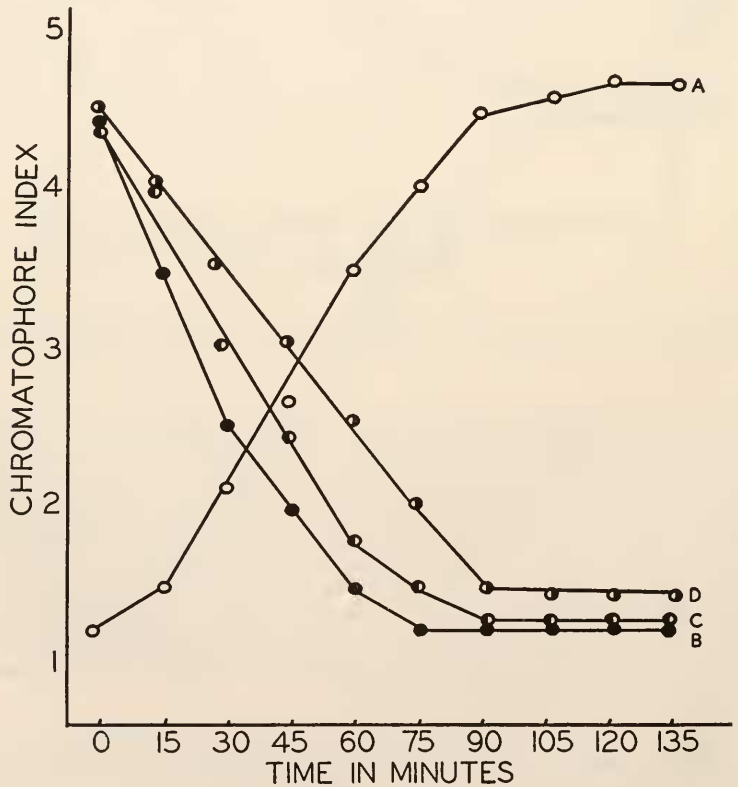


FIGURE 9. Further demonstration of the mutual antagonism existing between two chromatophorotropins of *Trachelipus*. A—dispersing factor alone (conc. 5). B—concentrating factor alone (conc. 5). C—concentrating and dispersing factor in a ratio of 4:1. D—concentrating and dispersing factor in a ratio of 3:2.

system, with the exception of the optic tracts and cerebral ganglia, does not contribute to the dispersing activity to any appreciable degree.

When the effects of very low concentrations of extracts were studied it was found that extracts of sinus glands, optic tracts and cerebral ganglia (conc. 0.25) produced only a dispersing effect, irrespective of the initial state of the chromatophores. On the other hand, extracts of the sinus glands plus the entire nervous system (conc. 0.25) exerted both a concentrating and a dispersing influence (see Fig. 8) dependent upon the initial state of the chromatophores. These results give support to the evidence that two factors are involved, since both types of response were elicited by the same extract when employed in low concentrations. The differential effects observed would not seem to be due to differing concentrations of the same substance.

In an attempt to determine the degree of antagonism existing between the two factors, extracts were prepared in a concentration of 5, of the concentrating factor and of the dispersing factor. These extracts were then mixed in various concentrations (4:1 and 3:2) and their effect determined. Figure 9 illustrates the responses which were obtained. It can be seen that when the concentration of the concentrating factor exceeds that of the dispersing factor, only the effect of the former is evident. The antagonism shown to exist between the two factors demonstrates that high concentrations of either factor will inhibit the effects of the more dilute factor. When both factors are present in small quantities, each produces a weak response and the chromatophores affected reach an intermediate condition.

5. Responses of Trachelipus

Since considerable technical difficulty is associated with the injection of *Trachelipus,* the results obtained from these experiments cannot be considered as conclusive. Due to the decreased viability of the animals and to the difficulty of observation following injection, the responses could not be readily interpreted. In general, the following effects were initiated.

Injection extract	Response of *Trachelipus*
Cambarus sinus glands	darkening
Trachelipus sinus glands	lightening
Trachelipus nerve cords	darkening

Although the results obtained must be considered as inconclusive, they suggest that the responses of the chromatophores of *Trachelipus* are directly opposite to those of the red chromatophores of *Cambarus.*

Discussion

The sinus glands of *Trachelipus* have been shown to contain a substance inducing a dispersion of the red pigment of the decapod *Cambarus,* and possibly a concentration of the melanin of the isopod *Trachelipus.* A dispersion of the red pigment of the decapod *Leander* following injection with extracts of the entire heads of terrestrial isopods *Mesidothea, Porcellio,* and *Oniscus* has previously been reported by Ståhl (1938). On the other hand, concentration of the melanin of

the isopod *Idothea* following injection of head extracts of the terrestrial isopod *Armadillidium* was observed by Okay (1945).

The results presented here indicate that the sinus glands at least of terrestrial isopods, although anatomically very similar to those of decapods, elaborate a substance inducing effects opposite to those elicited by extracts of the eyestalks of *Palaemonetes*-like decapods.

It has been shown by previous work (Koller and Meyer, 1930; Suneson, 1947; Carstam and Suneson, 1949) that eyestalk extracts of decapod Crustacea induce melanin dispersion in certain isopods. Evidence presented in this work, while only suggestive, indicates that the response in *Trachelipus* to *Cambarus* extracts is therefore like that of *Idothea*. In this respect, the isopods examined resemble the brachyuran Crustacea. This suggests an apparent difference in the reactive system as well as in the chromatophorotropins elaborated by the sinus glands of the two groups. However, there would appear to be a functional similarity, since extracts of the sinus glands of either group, when assayed upon their normal reactive system, are found to induce a concentration of the predominant pigment type.

The presence of a second and antagonistic hormone controlling the melanophores of isopods was suggested by Smith (1938) working with *Ligia oceanica*. He concluded that the background responses of *Ligia* are dependent upon the presence of two antagonistic hormones, one inducing a dispersion of the melanin, and the other a concentration of the same pigment.

Extracts of the connectives and thoracic cord of *Trachelipus* were found to induce a concentration of the red pigment of *Cambarus*, and possibly a dispersion of the melanin of *Trachelipus*. This factor, which induces an effect opposite to that produced by extracts of the sinus gland, is almost uniformly distributed throughout the connectives and ventral nerve cord.

A mutual antagonism has been shown to exist between the two chromatophorotropins of *Trachelipus*, at least in respect to their effect upon the red pigment of *Cambarus*. The direction and magnitude of the response is the result not only of the concentration of each factor, but, more directly, of the ratio between these concentrations. In this way a sufficient excess of either factor prevents the more dilute factor from eliciting a response. On the other hand, very dilute extracts known to contain small quantities of both factors were observed to induce responses in either direction dependent upon the initial condition of the chromatophores. The chromatophores perfused with these extracts attained a final condition of intermediate dispersion. Extracts prepared from the entire nervous system, including the sinus glands, have been shown to induce only a dispersing effect at high concentrations, while weak responses in both directions were elicited by dilute extracts. The rate of response is determined to a considerable degree by the concentration of the extract, but again is more closely correlated with the ratio between the two antagonistic factors present within a given extract. In regard to this point, it should be noted that the subesophageal ganglion and the connectives are located within the head of terrestrial isopods and, therefore, extracts prepared from the entire head would be expected to contain both factors. Due to the observed antagonism between the two factors, the weak activity of the optic tracts and cerebral ganglia does not necessarily preclude the possibility that both factors may be present within these tissues.

The effect which these observed properties of the two chromatophorotropins have upon the normal responses of the isopod must remain, for the present, hypothetical. It is interesting to note, however, the close correlation between the two chromatophorotropins of *Trachelipus*. Should this second factor be subsequently shown to exist within the nervous systems of isopods in general, the dispersion of the melanin observed to occur following the stimulation of the dorsal ocelli (Smith, 1938) may be due to the elaboration of high titers of this substance.

The identity or lack of identity of this factor with the *Crago*-darkening hormone found to be uniformly distributed throughout the central nervous system of *Idothea* (Brown and Saigh, 1946) also remains to be determined.

Although the observed chromatic responses of *Trachelipus* were slight, they appear to follow the same pattern as those observed in other species of isopods. However, the slight physiological color changes induced either by alterations in light stimulus or by the injected extracts could not be the sole explanation of the wide variations in color existing among these animals. Morphological color changes must necessarily assume an important role in the coloration of the adult animal. On the other hand, the ability of larval *Trachelipus* to exhibit strong physiological responses to light suggests that the loss of reactivity of the pigmentary system is a function of the progressive fusion of the chromatophore network with age.

In general, it can be stated that the progressive decrease in the reaction of the pigmentary system of *Trachelipus* is not accompanied by a corresponding loss of the chromatophorotropins of the sinus glands and central nervous system. It is possible that the hormones controlling physiological color changes may also be of functional significance in the control of the morphological color changes observed in these animals.

Summary

1. The terrestrial isopod *Trachelipus rathkei* was found capable of exhibiting weak physiological color changes in response to changes in background. Under the stimulus of diffuse light, animals maintained upon a light background became lighter than animals maintained in the dark. Under the stimulus of more intense light, the animals darkened even upon a light background.

2. Structures showing a marked resemblance to the sinus glands of decapods are located at the distal end of each optic tract and agree in position with the sinus glands of other isopods.

3. Extracts of the sinus glands of *Trachelipus* induce a strong dispersion of the red pigment of *Cambarus*.

4. Extracts of the optic tracts or cerebral ganglia induced a weak dispersion of the red pigment of *Cambarus*, while extracts of the connectives or any segment of the thoracic cord induced a strong concentration of the same pigment.

5. A comparison of the influence of change in concentration upon the effectiveness of the two chromatophorotropins indicates that above a minimal concentration, the rate of response is only slightly increased, whereas the magnitude of response remains unaltered. Below this level, both the rate and magnitude of response decline with decreasing concentrations.

6. An antagonistic action was found to exist between the two chromatophorotropins such that high concentrations of either factor prevented the more dilute

factor from exerting its influence. When very dilute extracts known to contain small quantities of both factors were assayed, both responses were elicited, such that the chromatophores assumed a condition of intermediate dispersion.

7. The response of *Trachelipus* to injected extracts proved inconclusive. The initiated responses suggest that the reactions of the pigmentary system of *Trachelipus* to injected extracts are opposite to those of *Cambarus*.

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